REMARKS

It is respectfully requested that this application be reconsidered in view of the above amendments and following remarks and that all of the claims under examination be allowed.

Interview Summary

Applicants wish to thank Examiners Housel and Hill for extending the courtesy of discussing this application with Applicants' representatives Janis Fraser and Ping Hwung in a telephone interview on April 13, 2005. All the pending claims under examination were discussed in view of the references cited in the Office Action. Applicants contended that the claimed invention possesses unexpected benefits, and presented an additional reference, Morita et al.[1] (attached herewith as Exhibit A), in support of this notion. Applicants further contended that the Office Action does not establish a *prima facie* case of obviousness. The Examiners tentatively agreed that Morita et al. demonstrate unexpected benefits of the subject matter of claims 3 and 4. Thus, the Examiners suggested that Applicants cancel claim 1 and rewrite claims 3 and 4 as independent claims.

With respect to claim 11 and its dependent claims, the Examiners agreed that they are not obvious, and that submission of an appropriate declaration would overcome the enablement rejection.

As this response is prepared according to these discussions and suggestions, Applicants submit that the currently pending claims are in condition for allowance.

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[1] Morita et al. Plat-E: an efficient and stable system for transient packaging of retroviruses. Gene Therapy 7:1063-1066 (2000).

Claim Amendments

Claims 1, 6-8 and 12-15 have been canceled without prejudice or disclaimer.

Claims 3 and 4 have been rewritten as independent claims by incorporating all the claim elements from their respective base claims (since the unamended claim 4 should have properly depended from claim 1, not claim 3, claim elements of claim 1 were incorporated).

Applicants submit that all claim amendments presented herein or previously are made solely in the interest of expediting allowance of the claims and should not be interpreted as acquiescence to any rejections or ground of unpatentability. Applicants reserve the right to file at least one continuing application to pursue any subject matter that is canceled or removed from prosecution due to the amendments.

Rejection Under 35 U.S.C. §112

Claim 11 stands rejected under 35 U.S.C. §112, first paragraph, on the ground of non-enablement. Specifically, the Office Action requires a statement regarding the public availability and conditions of deposit of the cells claimed in claim 11. In response, a Declaration Of Masao Haruna Under 37 C.F.R. §§1.801-1.809 that contains the required statement is submitted herewith. Accordingly, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. §103

Claims 1, 3-8, 11-14 and 16-28 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable in view of Klatzmann et al. (WO 98/02529, equivalent to U.S. Patent Application Publication No. 2002/0123146 A1; hereinafter "Klatzmann") and Hobbs et al. (Biochem. Biophys. Res. Comm. 252:368-372, 1998; hereinafter "Hobbs"). Claims 1, 6-8 and 12-14 have been canceled. With respect to the remaining claims, this rejection is respectfully traversed on a number of grounds. First, claim 11 is directed to specific cells that are defined by particular deposit accession numbers, and claims 27 and 28 are directed to methods of using these cells. These cells and methods are plainly not obvious in view of the cited references. Second, there is no motivation for a skilled artisan to combine Klatzmann and Hobbs and modify the combined

teachings to arrive at the claimed invention. Third, the claimed invention provides an unexpected benefit that could not have been predicted from the cited references. Fourth, the cited references do not teach or suggest all the claim elements of claims 16 and 22.

These reasons are elaborated below.

A. Claims 11, 27 and 28

Claim 11 is directed to a cell specified by Accession No. FERM BP-6737 or FERM BP-6977 as deposited at the National Institute of Bioscience and Human-Technology in Japan. A skilled artisan does not have any motivation, suggestion, or reasonable expectation of success to combine Klatzmann and Hobbs and modify the combined teachings to arrive at the cell specifically claimed in claim 11. The combined references also do not teach or suggest each and every element of the claimed cell. Therefore, claim 11 is clearly not obvious in view of the cited references. Similarly, claims 27 and 28, directed to methods of using the claimed cell, are also not obvious.

B. Lack of motivation

Claim 3 is directed to a 293T cell useful for the production of retroviruses, wherein the cell contains an expression construct comprising DNA encoding gag, pol and env retroviral structural proteins operably linked downstream of an EFlα promoter. Each of the three coding sequences can be driven by a separate EFlα promoter, or two or even all three can be linked in tandem to the same EFLα promoter sequence. Claim 4 is directed to a 293T cell useful for the production of retroviruses by expressing retroviral structural proteins gag, pol and env, wherein the cell comprises a first expression construct expressing gag and pol from an EFlα promoter, and a second expression construct expressing env from an EFlα promoter. All other rejected claims (except claims 11, 27 and 28) depend from claim 3 or 4.

Klatzmann teaches so-called packaging cells that can be used to produce defective viruses carrying a transgene, including packaging cells that express the gag, pol or env gene. The

reference does not teach use of the EFlα promoter, nor use of 293T cells. Hobbs teaches a bicistronic vector that employs the human EFlα promoter in 293T cells, but does not mention retroviral proteins or packaging cells. The Office Action takes the position that because Klatzmann allegedly discloses a need for a strong promoter and Hobbs teaches that EFlα is a strong promoter, a skilled artisan would have been motivated to combine Klatzmann with Hobbs and modify the teachings to arrive at the claimed invention.

Applicants respectfully disagree with this conclusion, particularly with respect to the currently pending claims. As amended, the currently pending claims require that all three structural proteins, gag, pol and env, be expressed from the human EFlα promoter. Klatzmann teaches that gag and pol can be expressed using an LTR promoter (Paragraph 43), while env may be expressed from a strong promoter or inducible promoter (Paragraphs 54-55). Given the express teaching of Klatzmann that one should use an LTR promoter for gag and pol, a skilled artisan would not have been motivated to replace the promoter that Klatzmann uses for gag and pol with the human EFlα promoter, which is not an LTR promoter or even related to an LTR promoter.

Accordingly, a skilled artisan would not have been motivated to combine Klatzmann and Hobbs, and modify the combined teachings to arrive at the claimed invention. Thus, a *prima facie* case of obviousness has not been established, and withdrawal of this rejection is respectfully requested.

C. Unexpected properties

The claimed packaging cells have the dual, unexpected benefits of (1) high infection efficiency, which indicates the titer of viruses produced by a packaging cell, and (2) long-term stability (e.g., Examples 8 and 10, discussed in detail in the response filed September 9, 2004). Nevertheless, the Office Action alleges at the bottom of page 4:

> The arguments of unexpected results are not commensurate with the claims. The claims are drawn to cells and a method of producing a retrovirus and not to a method of expressing proteins for a period of time.

Applicants submit that the unexpected results are commensurate with the claims. High infection efficiency and long-term stability are properties of packaging cells and methods of producing retroviruses, rather than of methods of expressing proteins for a period of time. For example, Example 8 (pages 14-15) discloses that packaging cells prepared according to the present invention ("PLAT-E" cells) produced retrovirus with an infection efficiency of 90% even after two months of passage; in contrast, prior art packaging cells (BOSC23) had dropped down to an infection efficiency of only 23% after two months. This point is also well-illustrated in a post-filing date publication by the inventors that describes the present invention (Morita et al., Exhibit A). Figure 3 of Morita et al. shows that the infection efficiency of PLAT-E was well above prior art cells (BOSC23 and Phoenix-E) for many months. These are obviously very desirable properties for cells that are created to produce viruses.

Such superior properties were unexpected. In the claimed invention, gag, pol and env are expressed from the human EFlα promoter. In the prior art cells BOSC23, gag, pol and env are expressed from the MuLV-LTR, while the RSV and CMV promoters are used in the Phoenix-E cells. Figure 1 of Morita et al. illustrates that the relative strengths of these promoters are in the following order: CMV promoter > EFlα promoter > RSV promoter >> MuLV-LTR. Since the MuLV-LTR is by far the weakest among these promoters, it would have been expected that the cells using this promoter (BOSC23 cells) should produce the lowest infection efficiency, while the Phoenix-E and PLAT-E cells should be comparable to each other in infection efficiency. Surprisingly, BOSC23 produced significantly more viruses than Phoenix-E, while the infection efficiency of PLAT-E cells was much higher than that of either BOSC23 or Phoenix-E (Figure 3 of Morita et al.). Thus, the infection efficiency of PLAT-E is unexpectedly high. It was also unexpected that the infection efficiency of PLAT-E remained high for several months, while those for BOSC23 and Phoenix-E dropped rapidly with time (Figure 3). Neither of these benefits of PLAT-E could have been predicted based on the relative strength of the EFlα promoter compared to that of the other three promoters.

Accordingly, the claimed invention provides surprising and unexpected benefits that are probative of non-obviousness, and withdrawal of this rejection is respectfully requested.

D. Claims 16 and 22

In addition to the reasons set forth above, which apply to all of the claims, Applicants submit that there is an additional ground for recognizing the non-obviousness of claims 16 and 22.

To properly issue a rejection under 35 U.S.C. §103, the USPTO bears the initial burden of establishing a *prima facie* case of obviousness by meeting certain criteria. One of these criteria is the requirement that the cited references must teach or suggest all the elements of the claimed invention. Claims 16 and 22 depend from claims 3 and 4, respectively, further requiring that a Kozak's consensus sequence be located upstream of a translation initiation codon of the DNA encoding the retroviral structural proteins in the expression construct. Since neither Klatzmann nor Hobbs teaches such a sequence, the cited references do not teach or suggest all the claim elements of the claimed invention. Therefore, the rejection with respect to claims 16 and 22 should be withdrawn for this reason alone.

Conclusions

For the reasons set forth above, Applicants submit that the claims of this application should be allowed. Reconsideration and withdrawal of the Examiner's rejections are hereby requested. Allowance of the claims under examination in this application is earnestly solicited.

In the event that a telephone conversation could expedite the prosecution of this application, the Examiner is requested to call the undersigned at (617) 542-5070 or the undersigned's associate, Ping Hwung, at (650) 839-5044.

Enclosed is a $450 check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: May 2, 2005

Reg. No. 44,164

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EXHIBIT A

Gene Therapy (2000) 7, 1063–1066

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VIRAL TRANSFER TECHNOLOGY

BRIEF COMMUNICATION

Plat-E: an efficient and stable system for transient packaging of retroviruses

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A potent retrovirus packaging cell line named Platinum-E (Plat-E) was generated based on the 293T cell line. Plat-E is superior to existing packaging cell lines regarding efficiency, stability and safety. The novel packaging constructs utilized in establishment of Plat-E ensure high and stable expression of viral structural proteins. Conventional packaging constructs made use of the promoter of MuLV-LTR for expression of viral structural genes gag-pol and env, while our packaging constructs utilized the EF1α promoter, which is 100-fold more potent than the MuLV-LTR in 293T cells in combination with the Kozak's consensus sequence upstream of the initiation codon resulting in high expression of virus structural proteins in Plat-E cells. To maintain the high titers of retroviruses under drug selection pressure, we inserted the IRES (internal ribosome entry site) sequence between the gene encoding gag-pol or env, and the gene encoding a selectable marker in the packaging constructs. Plat-E cells can stably produce retroviruses with an average titer of 1 × 10⁷/ml for at least 4 months. In addition, as we used only the coding sequences of viral structural genes to avoid inclusion of unnecessary retrovirus sequences in the packaging constructs, the probability of generating the replication competent retroviruses (RCR) by recombination can virtually be ruled out. Gene Therapy (2000) 7, 1063–1066

Keywords: *packaging cell; retroviruses; ecotropic; EF1α promoter*

Retroviral vectors and packaging cells are important tools for gene transfer applications. Introduction of retroviral vectors containing the gene of interest into suitable packaging cells enables production of infectious retroviruses, and these particles can infect target cells and stably transmit the gene of interest into chromosomes. In conventional strategies, stable high producers of a retrovirus vector harboring a gene of interest were established by transducing the retrovirus construct into NIH3T3-based packaging cells such as PA317,[1] and 2–3 months were usually needed to acquire high producers. Pear *et al*[2] developed a unique packaging system by which high titer retroviruses can be obtained in 3 days by transient transfection. The expression of viral structural genes was driven by the MuLV LTR in Bosc23 cells. For transient transfection, the combination of Bosc23 cells and the pMX-neo vector[3] produced $1–3 \times 10^6$/ml viruses, assessed based on the number of neomycin resistant colonies of the infected NIH3T3 cells (data not shown). Since Bosc23 cells carry the large T antigen, we attempted to increase titers of the retroviruses by introducing the SV40 origin to the pMX vector for amplification of the vector. However, this proved unfeasible (data not shown), suggesting that the limiting factor was the expression level of the viral structural proteins in the packaging cells. Bosc23 was obtained by cotransfection of the plasmid encoding *gag-pol* together with the plasmid encoding the hygromycin-resistant gene and the plasmid encoding *env* together with the plasmid encoding another selection gene GPT (guanine phosphoribosyl transferase), one after the other. Therefore, expression of selectable markers did not guarantee the expression of *gag-pol* or *env* genes, which may account for the instability of the cells in producing high-titer retroviruses.

A similar packaging cell line Phoenix-E[4] has also been developed. In Phoenix-E cells, the plasmids encoding the *gag-pol* and *env* genes were cotransfected with selection markers, which did not warrant the stable expression of the *gag-pol* and *env* genes in the selection drug, hygromycin and diphtheria toxin, respectively. There were several improvements in the Phoenix-E cells when compared with Bosc23 cells. First, the RSV and CMV promoters, which are much stronger than MuLV-LTR in 293T cells, were used to express the *gag-pol* and *env* genes, respectively. Second, the internal ribosome entry site (IRES[5]) sequence was used to express *gag-pol* and a cell surface marker CD8 simultaneously which enables sorting of high expressers of the *gag-pol* gene. However, one needs to sort cells from time to time to maintain the expression levels of the *gag-pol* gene.

To design a packaging cell line which can stably produce retroviruses with high titer, we searched for a strong promoter to drive expression of viral structural proteins in 293T cells using the FACS-GAL assay.[6] Among seven promoters tested, the EF1α and CMV promoters induced high expression of *lacZ* (Figure 1). The activities driven by these promoters were 100-fold higher than those by LTR utilized in Bosc23 cells, and even exceeded those by SV40 and SRα promoters, which enable amplification of vectors in 293T cells expressing

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Received 3 March 2000

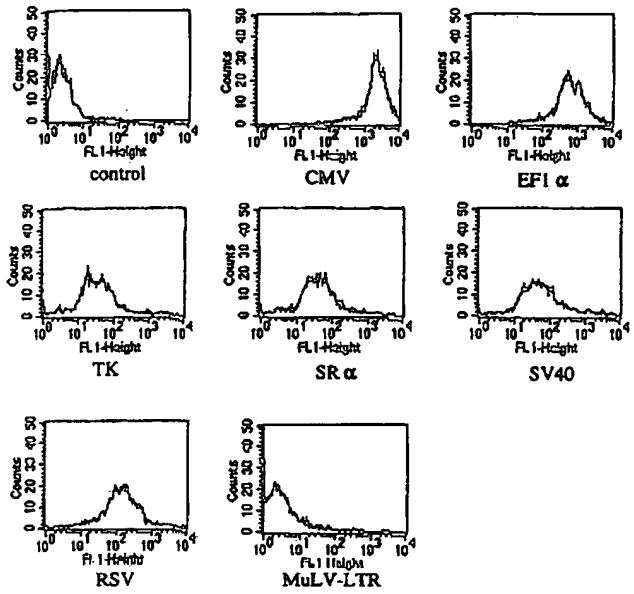

Figure 1 Activities of various promoters in 293T cells The activities of the seven promoters, SV40, SRα, EF1α, RSV, TK, MuLV LTR and CMV were evaluated by expression of lacZ under the control of each promoter in 293T[7] cells by the FACS-GAL assay as described.[6] Briefly, cells (1 × 10⁶) transfected with each promoter construct were suspended in 50 μl of phosphate buffered saline (PBS), then incubated for 5 min at 37°C. FDG (fluorescein di-β-D-galactopyranoside; Molecular Probes, Eugene, OR, USA) was dissolved in distilled water, warmed at 37°C and 50 μl of 2 mM FDG solution was added to 50 μl of cell suspension. After 1 min of incubation at 37°C, 1 ml of PBS was added followed by incubation on ice for 2 h. To stop the reaction, 20 μl of 50 mM PETG (phenylethyl-β-D-thiogalactoside; Sigma, St Louis, MO, USA) was added, and the preparation was placed on ice until being subjected to FACS analysis.

the SV40 large T antigen. Because we thought that the promoters of housekeeping genes were more suitable than the viral promoters for driving stable gene expression in mammalian cells, we used the EF1α promoter to express the viral structural proteins in 293T cells (Figure 2) In addition, IRES was inserted between the *gag-pol* or *env* gene and the selection marker in the packaging constructs described here. Therefore, expression of the selection marker is a direct reflection of *gag-pol* or *env* expression in the same cells.

Packaging constructs pEnv-IRES-puro^r and pGag-pol-IRES-bs^r, which were constructed as described above, were sequentially transfected into 293T cells and 50 subclones resistant to both puromycin and blasticidin were isolated. Among 50 subclones, clone 1 named Platinum-E (Plat-E) produced the retrovirus which had the highest infection efficiency and was used for further analysis. The titer of the retroviruses was about 1×10^7/ml when tested on NIH3T3 cells, using serially diluted virus supernatants of Plat-E cells transfected with pMX-lacZ (data not shown). We next compared early passages of Plat-E cells with those of Bosc23 cells and Phoenix-E cells with regards to long-term stability to produce high-titer retroviruses by transient transfection (Figure 3). Culture conditions of the three packaging cell lines were as follows: Bosc23 cells were grown in DMEM with 10% fetal bovine serum containing the GPT selection reagents as indicated by the manufacturer (Specialty Media, Lavallette, NJ, USA). Phoenix-E cells were sorted by FACS for

pGag-pol-IRES-bs^r

pEnv-IRES-puro^r

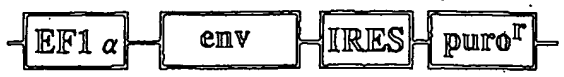

Figure 2 Schematic diagrams of packaging constructs. The packaging constructs used for development of Plat-E are shown. The fragment carrying the selectable marker, the blasticidin resistant gene (bs^r) or the puromycin resistant gene (puro^r), was obtained by PCR using a pair of oligonucleotides (for bs^r: 5'-AAAACATTTAACATTTCTCAACAAG-3', 5'-ACGCGTCGACTTAATTTCGGGTATATTTGAGTG-3', for puro^r: 5'-ACCGAGTACAAGCCCACG-3', 5'-ACGCAGATCTTCAGGCACCG GGCTTG-3'), and were inserted in the NcoI and SalI site (for bs^r), or in the NcoI and BglII site (for puro^r) of pMX-IRES-EGFP.[8] The fragments containing the IRES sequence and either of bs^r and puro^r were excised from the vector by NotI and SalI for bs^r, or NotI and BglII for puro^r, respectively. The viral structural genes, gag-pol and env were amplified by PCR, using the MoMuLV genome as a template, and the oligonucleotide primers were used as follows Each primer contains either the EcoRI site or the NotI site (underlined) and the 5' primers also contain a Kozak's consensus sequence GCCGCCACC located upstream of the initiation codon. gag-pol: 5'-CGAATTCGCCGCCACCATGGGCCAGACTGTT ACCACTCCCTTAA-3'; 5'-TACGCGGCCGCTCTGAGCATCAGAA GAA-3'; env: 5'-cGAATTCGCCGCCACCATGGCGCGTTCAACGCT CTCAAAA-3'; 5'-TACGCGGCCGCTATGGCTCGTACTCTAT-3'. The resulting PCR fragments were digested with the EcoRI and the NotI fragment. Finally, the fragment containing the viral structural genes, and the fragment containing the IRES sequence and the selection marker were inserted downstream of the EF1α promoter in the pCHO vector, a derivative of pEF-BOS.[9] For construction of the pGag-pol-IRES-bs^r, pCHO was digested with BamHI, and converted to a blunt end by Klenow reaction, and then ligated with SalI linker (Stratagene, La Jolla, CA, USA). The EcoRI–NotI fragment of gag-pol, and the NotI–SalI fragment of IRES-bs^r were inserted into the EcoRI and the SalI site of pCHO by triple ligation. To construct pEnv-IRES-puro^r, pCHO was digested with EcoRI and BamHI, and the EcoRI–NotI fragment of env, and the NotI–SalI fragment of IRES-puro^r were inserted into the EcoRI and the BamHI sites of pCHO. Packaging constructs pEnv-IRES-puro^r and pGag-pol-IRES-bs^r were sequentially transfected into 293T cells using Fugene (Boehringer Mannheim, Germany) according to the manufactuer's recommendations. One day after transfection with pEnv-IRES-puro^r, 293T cells were selected in DMEM containing 1 μg/ml puromycin. The selected cells were then transfected with the pGag-pol-IRES-bs^r vector, and subcloned in the presence of puromycin and blasticidin (10 μg/ml). The selected clones were tested for their potential to produce retroviruses. EF1α, EF1α promoter; IRES, internal ribosome entry site; bs^r, blasticidin resistant gene; puro^r, puromycin resistant gene.

fetal bovine serum containing hygromycin (300 μg/ml) and diphtheria toxin (1 μg/ml) for 1 week, then cells were transferred to DMEM with 10% fetal bovine serum without hygromycin and diphtheria toxin. Plat-E cells were always maintained in DMEM with 10% fetal bovine serum containing blasticidin (10 μg/ml) and puromycin (1 μg/ml). On one hand, infection efficiency of retroviruses produced from Bosc23 was decreased within 3 months, and that of retroviruses produced from the Phoenix-E cells also decreased in time (Figure 3). On the other hand, Plat-E produced retroviruses with an infection efficiency greater than 75% with a titer of about 1×10^7/ml for at least 4 months under drug selection pressure.

To compare the expression level of *gag-pol* and *env*

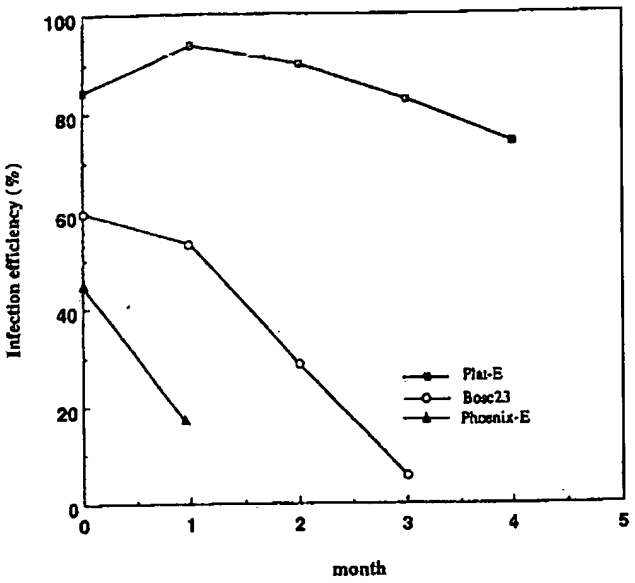

Figure 3 Long-term stability of Plat-E in producing high titer retroviruses. The infection efficiencies of Ba/F3 cells using retroviruses derived from pMX-GFP produced by Plat-E, Bosc23 and Phoenix E were examined at the indicated times. pMX-GFP was constructed as follows The GFP fragment was excised from the pEGFP-N1 vector (Clontech, Palo Alto, CA, USA) by EcoRI and NotI, and was inserted into the EcoRI–NotI site of the pMX vector[3] Transfection and infection were performed as described[10] except that we used Fugene (Boehringer Mannheim) instead of LipofectAmine (Gibco-BRL, Rockville, MD, USA).

lines, Northern blot analysis was done using the cells cultured for 3 weeks. The expression levels of *gag-pol* and *env* mRNA was four-fold and 10-fold higher, respectively, in Plat-E cells than in the other packaging cell lines (Figure 4a). The RT activity in the cell lysate was also analyzed. Plat-E produced at least twice more RT activity when compared with Bosc23 and Phoenix-E cells (Figure 4b). In addition, the expression level of *env* protein was much greater than that of Bosc23 and Phoenix-E (Figure 4c) when evaluated by antibody staining raised against the env gene product.

As the retroviral structural genes were encoded on the two different plasmids, three recombination events are necessary to generate the replication competent retroviruses (RCR). In addition, the probability of recombination was minimized by using only the coding sequence of *gag-pol* and *env* genes isolated by PCR from MuLV genome in the packaging constructs. In fact, production of RCR was tested by the XC plaque assay,[13] and no RCR was detected from Plat-E cells after transfection of pMX-GFP As for a positive control, a supernatant of MoMuLV-infected C3H2K cells (a gift from Dr Hoshino) was used after serial dilutions, and the viral titer of the wild-type MoMuLV produced from C3H2K cells was estimated as 1×10^4/ml

In conclusion, we report here a stable retrovirus packaging cell line Plat-E which has several advantages over the existing packaging cell lines. First, the EF1α promoter in the packaging constructs, in combination with the Kozak's consensus sequence, allows production of retroviruses with a titer of 1×10^7/ml. Second, a bicistronic vector carrying the IRES sequence was used in the packaging constructs to ensure stable expression of the viral

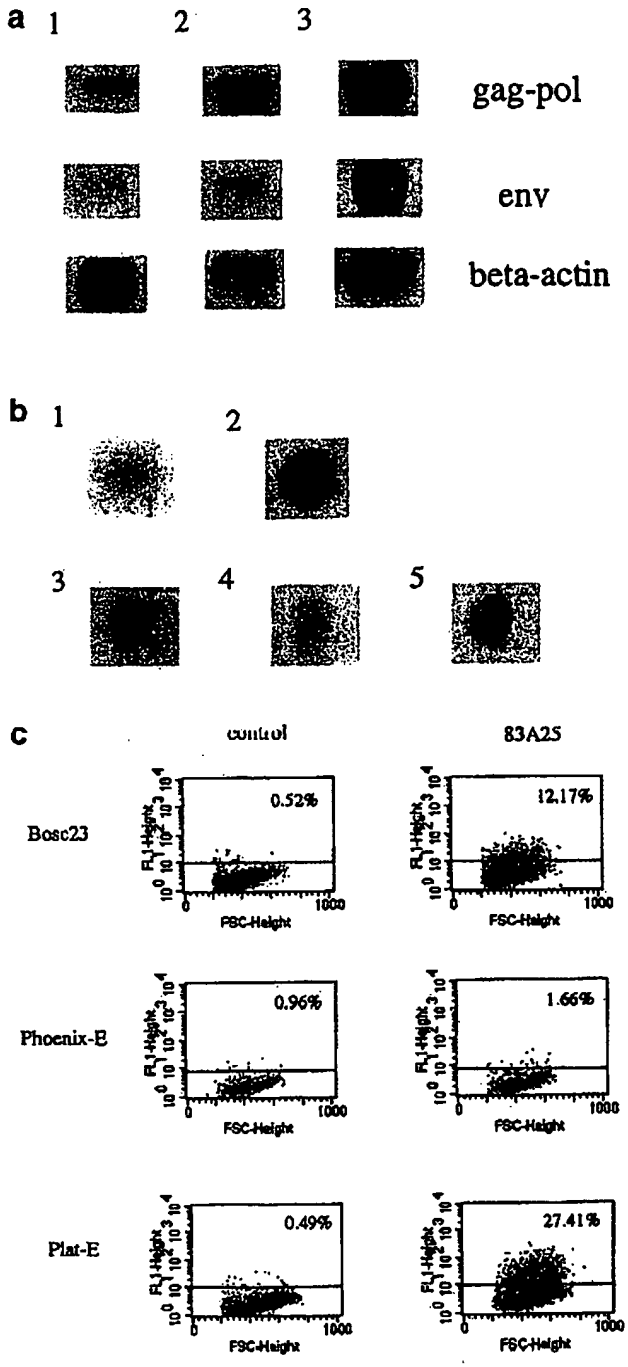

Figure 4 Comparison of gag-pol and env expression in Bosc23, Phoenix-E and Plat-E (a) Northern hybridization of gag-pol and env. Expression of gag-pol and env in Plat-E (3) was compared with that in Bosc23 (1) and Phoenix-E (2) by Northern hybridization. The probes used were the EcoRI–NotI fragment of pGag-pol-IRES-bsr and pEnv-IRES-puro. (b) RT assay of cell lysate of Bosc23, Phoenix-E and Plat-E. Cell lysate of 293T cells was used as a negative control (1), 1 ng of HIV RT was mixed with the cell lysate of 293T cells (2), and was used as a positive control. RT activities derived from Bosc23 (3), Phoenix-E (4), Plat-E (5) were measured as described.[11] (c) Expression of env in Bosc23, Phoenix E and Plat-E cells. The expression of env was determined by cell surface fluorescence of Bosc23, Phoenix-E and Plat-E using the rat monoclonal antibody raised against the env proteins termed 83A25.[12] Staining procedure was performed as described[12] and then subjected to FACS analysis. As a control, these cells were stained only with the second antibody (FITC-conjugated goat anti-rat IgG as second antibody).

makes it possible to maintain the titer of retroviruses derived from the Plat-E cells by simply culturing the cells in the presence of selection drugs. Finally, to lessen the possibility of generation of RCR, the minimum virus sequences were used in the packaging constructs Thus, Plat-E cells can stably produce helper-free retroviruses at high titers for a long time.

Using retroviruses produced by Plat-E cells, we can efficiently transfer genes to many different cells including cells in primary culture such as T cells and mast cells (data not shown). Recently, it has been reported that by introducing the coding region of the polyomavirus early gene into the packaging cell lines, the titers of recombinant retrovirus produced by these cell lines were 10–100 times higher than those produced by the parent cell line.[14] Introduction of the polyomavirus early region into Plat-E may lead to more efficient production of retroviruses with high titer Plat-E is an ecotropic packaging cell line and generation of its amphotropic counterpart, the Plat-A cell line should prove useful in human gene therapy.

Acknowledgements

We would like to thank Dr Hiroo Hoshino (Department of Hygiene and Virology, Gunma University School of Medicine) for his kind gift of MoMuLV-infected C3H2K cells, and Dr Leonard H Evans (Laboratory of Persistent Viral Disease, National Institute of Allergy and Infectious Disease) for anti-Env antibody, and Dr Kunitada Shimotohno (Department of Viral Oncology, Institute for Virus Research, Kyoto University) for his valuable discussions. We also thank Ms Mariko Ohara for her providing language assistance. This work was supported in part by grant-in-aid from the Ministry of Education, Science, Sports, and Culture and the Ministry of Health and Welfare of Japan. The Department of Hematopoietic Factors is supported by Chugai Pharmaceutical Company Ltd

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